EFFECTS OF GAMMA IRRADIATION ON POPULATIONS OF SOIL INVERTEBRATES

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Abstract. Invertebrate animals living in soil or on moist plaster of Paris were irradiated with gamma rays from a cobalt source. Soil samples and cultures were adequately replicated and radiation doses ranged from 10 krads through 25, 50, 100 and 200 krads. The animals in culture included representative Oligochaeta, "Myriapoda," Acarina, Collembola and Insecta. Susceptibility to radiation varied greatly both between and within groups. Oribatid mites were most resistant, surviving up to 80 days after exposure to 200 krads; and isopods, which were most susceptible, were all dead 60 days after exposure to 10 krads. There was excellent correlation between activity and susceptibility to radiation.

Mortality/time curves were not linear even on a probit scale but were usually distinguishable into an initial steep curve and a shallow secondary one. Radiation dosages were positively correlated with percentage mortalities but not in a simple logarithmic or probit relationship.

Populations of all main groups of invertebrates in soil samples were regularly counted for three months after irradiation. Most decreased in numbers sigmoidally with time, but a few species eventually multiplied after a small dose of radiation, probably due to greater susceptibility of predator than prey. Animals were usually more susceptible to irradiation in cultures than in soil.

Introduction

The amount of environmental pollution by radioactive wastes and fallout increases annually. As the industrial use of atomic power becomes more widespread, the problem of radioactive waste disposal intensifies, and it becomes more likely that terrestrial ecosystems may become further contaminated and changed. At present, high level wastes (up to 500 curies of radioactivity per gallon of water) are usually discharged into the sea, but low level wastes (up to 100 millicuries per gallon) which come from factories, laboratories or hospitals may reach sewage beds or be otherwise dispersed into the soil. Smaller quantities of radioactivity reach the soil from aerial fallout or from decomposing plant and animal tissues which contain isotopic residues. With all these potential sources of terrestrial radioactivity, studies of the effects of radiation on the soil ecosystem seem essential. Soil invertebrates have important roles in this system by breaking down plant material and mixing it intimately into the soil and turning over and aerating the soil as they tunnel through it.

Investigations into the effects of radiation on invertebrate animals have been mainly confined to a few insect orders. Most studies have had secondary aims of using gamma radiation to control stored product pests, particularly beetles; to study its genetic and physiological effects on several test insects, especially *Bracon* and *Drosophila*; or to determine suitable dosages to sterilize economic insects which are then released to interfere with the mating and reproduction of natural populations. An adequate review of some of this work was made by O'Brien and Wolfe (1964). All of these studies have concerned the effects of radiation on collections of individuals of a single species, and few of the insects tested spend any part of their life in soil. Auerbach *et al.* (1957) did preliminary work on the effect of radiation on Collembola population growth in culture, but the only study of the effects of radiation on natural populations of invertebrates was that by Auerbach (1958) who indicated the possibility that treatment of an entire community with radiation may cause increase in numbers of some species, probably because a predator may be more susceptible to the radiation than its prey. Such increases in numbers of prey species have been shown to result commonly from the use of many pesticides in soil (Sheals 1956, Edwards 1960, 1965a, 1965b). To analyze such complex effects it is necessary to investigate the effects of the biocide both on single-species cultures of animals and on whole communities. The scope of the present work is to do this for a range of soil invertebrates. Furthermore, there have been no studies that have compared the effects of a range of doses of gamma radiation on many different invertebrate taxa simultaneously, to determine their relative susceptibility to the radiation.

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Methods

The experimental site at Rothamsted Experimental Station, England, was a mixed woodland soil rich in organic matter, which contained unusually large numbers of invertebrates representative of most of the groups which normally inhabit soil. The invertebrates were exposed to gamma irradiation either as mixed populations in intact soil cores or in single-species cultures kept on moist plaster of Paris. Soil cores for direct irradiation or for providing animals for the cultures were taken with a two-inch diameter sampling tool to a depth of 6 inches and put into aluminum cans slightly larger than the samples. The animals were extracted for the cultures as soon as possible in Tullgren funnels and collected in small vials with a layer of moist plaster of Paris on the bottom. The Tullgren funnels which were aluminum and steep-sided had soil containers six inches in diameter and four inches deep. One soil sample half-filled a soil container and the heat source above it gave a temperature gradient through the soil from 30° to 35°C at the top to 20°C at the bottom of the sample. Representative species of three families of Acarina and one each of Collembola, Diptera larvae, Coleoptera adults, Diplopoda, Isopoda and Oligochaeta (see Appendix) were chosen from the samples and kept in cultures on plaster of Paris. The microarthropods were cultured in vials one inch in diameter and one inch deep with a layer of moist plaster of Paris on the bottom. The larger invertebrate cultures were in petri dishes two inches in diameter also containing a layer of plaster of Paris. In the enchytraeid cultures, a thin moist layer of leaf litter was placed on the surface of the plaster, and one hundred and ten animals were put into each culture vessel. The other cultures each contained ten animals and small yeast pellets were added periodically as food for Collembola, Diplopoda, Isopoda and saprophagous Acarina.

Ten intact soil cores were taken close together from an area of uniform soil marked out into 12 adjoining quadrats each large enough to provide 60, two-inch diameter soil cores. Within each quadrat, samples were taken in six sets of ten, each set distributed evenly over the area. The samples, in aluminum cans were stored overnight at 5°C and irradiated the next day in a large package-irradiation plant at the Wantage Research Laboratory (AERE), Wantage, Berkshire, England. The source contained 300,000 curies of ^{60}Co in a rectangular frame, approximately seven feet long by two feet high with a gamma-dosage of 0.117 megarad per hour at a distance of one foot from the source. Samples and cultures were stacked in rows one above the other parallel to, and about one foot from the source and the doses of 10, 25, 50, 100, and 200 kilorads were varied by extending the times of exposure from about five to 100 minutes.

Five batches of single-species cultures each consisting of six cultures of wood lice, five cultures of oribatid and parasitid mites, three cultures of fly larvae, beetle adults and enchytraeid worms, and two cultures of uropod mites and millipedes were exposed to the five doses of radiation. One batch was not irradiated. At the same time five batches of soil samples consisting of ten samples from each of the 12 quadrats were irradiated with the five different radiation doses, within the aluminum cans in which they were collected. One similar control batch of samples was not irradiated although it was taken to the packaging plant with the other samples so that it had the same temperature changes as the irradiated ones.

The cultures and soil samples were kept in a constant temperature room at 15°C. All cultures were examined daily for the first week, and thereafter at intervals of 2 to 3 days, and all dead animals were counted and removed. Because the counts were spread over two months, mortality in the controls was inevitable. It was moderate for the Enchytraeidae, Isopoda and Oribatidae but heavy in the Parasitidae where there was much cannibalism although animal food was provided. After two months enough control animals of most groups had died to make further observations unprofitable.

The batches of soil samples were re-sorted into their original quadrat groupings so that every group contained five sets of ten samples each treated with one of the irradiation doses. Ten samples had not been irradiated. Every few days one group of samples was taken from the constant temperature room and placed in Tullgren funnels. These times were 1, 4, 10, 13, 19, 29, 42, 55, 62, 69 and 76 days after the samples were irradiated. The extraction of all the animals into vials containing 70% alcohol and 5% glycerine took three days. They were stored until they could be examined and categorized under the binocular microscope.

Results

The cumulative mortality counts in the cultures are summarized in Figs. 1 to 9; standard errors are not included to avoid confusing the graphs. The full data from the soil samples that were irradiated intact were too extensive to include here, but Table 1 gives the numbers of the various groups of invertebrates surviving 42 days after irradiation, expressed as a percentage of the control numbers. Table 2 lists the times taken for *all* the animals in ten soil samples to die, after exposure to 200 kilorads of radiation. These figures are approximated to the nearest ten days and are intended to show only the relative susceptibility of the animals to a large dose of radiation. The reason is that samples were examined at only 7 to 10 day intervals giving a range of about ±3 to 5 days in the estimate. Furthermore the Tullgren method of extraction imposed two limitations: firstly, animals which were not killed but only paralyzed would not emerge from the soil samples, and so were recorded as dead. Secondly the times quoted represent the intervals between irradiation and when the samples were placed in Tullgren funnels; but it takes three days for all animals to be extracted from a soil sample. So, with the majority leaving after 36 hours, this is likely to be

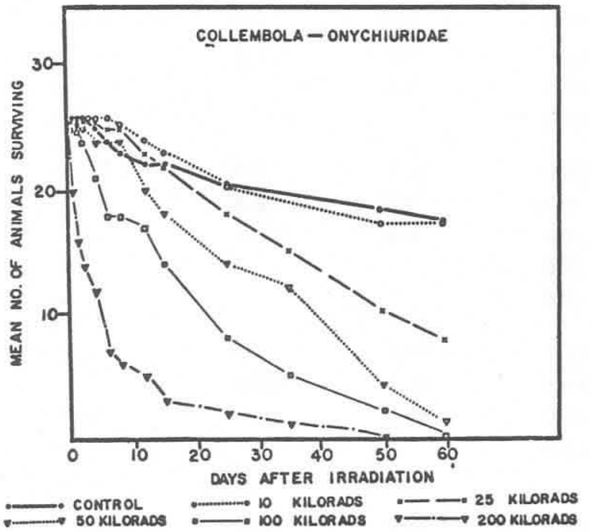

Fig. 1. Effects of different doses of gamma radiation on invertebrates in culture.

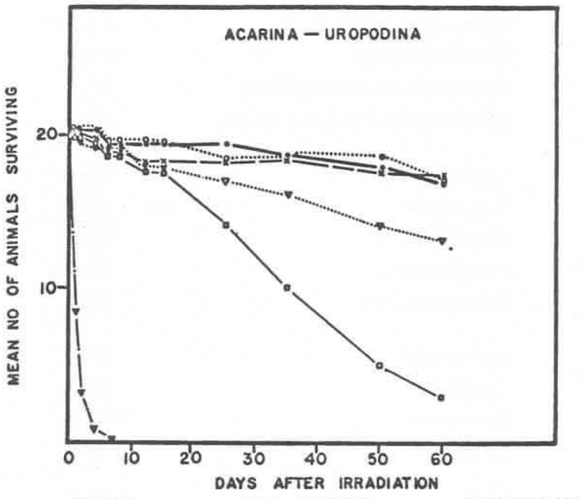

Fig. 2. Effects of different doses of gamma radiation on invertebrates in culture.

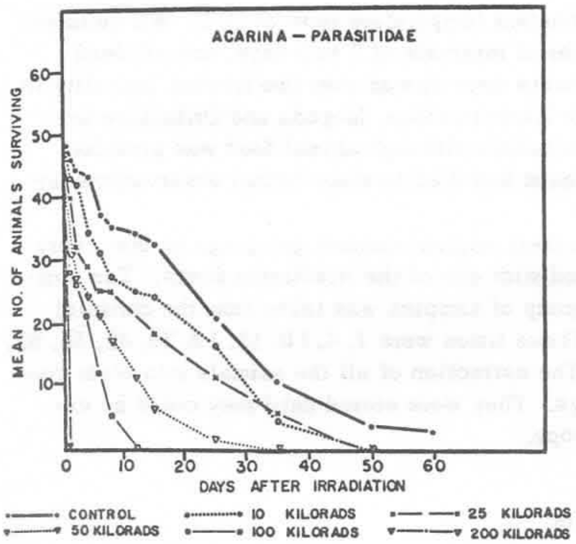

Fig. 3. Effects of different doses of gamma radiation on invertebrates in culture.

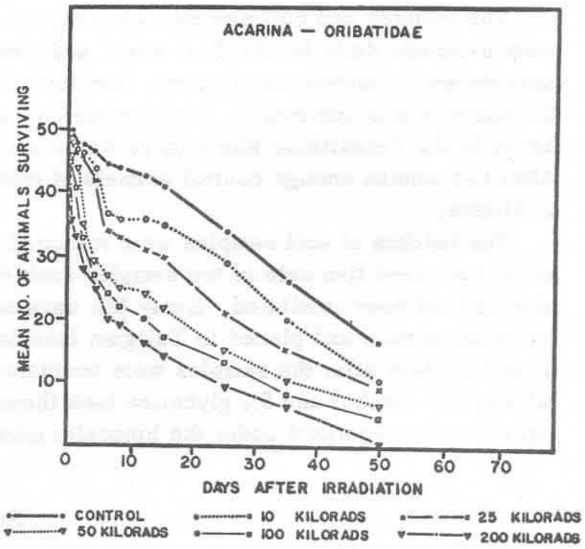

Fig. 4. Effects of different doses of gamma radiation on invertebrates in culture.

Table 1. Percentage survival in soil 42 days after gamma irradiation

Taxonomic Category	Percentage Surviving After 42 Days				
	10 kr	25 kr	50 kr	100 kr	200 kr
Nematoda	48.0	37.5	37.5	16.7	6.3
Lumbricidae	100.0	72.8	41.9	13.6	
Enchytraeidae	41.5	47.7	52.0	10.4	
Isopoda	12.5				
Oribatidae	90.5	48.4	16.3	16.3	8.4
Mesostigmata-Uropodina	118.0	69.0	7.0	7.0	1.0
Trombidiformes	114.0	15.7	2.7	1.2	
Mesostigmata-Parasitidae	37.6	12.6	0.8		
Mesostigmata-Rhodocaridae	40.0	15.0			
Onychiuridae	42.4	14.2	11.4	6.8	1.1
Poduridae	20.8	7.7	5.2	2.4	1.1
Isotomidae	38.9	5.8	1.0	0.2	0.1
Sminthuridae	50.0	1.9			
Entomobryidae	31.2	2.6			
Diplopoda	30.4	26.1	26.1	4.3	
Symphyla	88.9	116.4	61.1	50.0	
Chilopoda	125.0	50.0	50.0		
Pauropoda	34.4	13.8			
Diplura	30.0				
Diptera	48.4	43.0	35.5	19.4	4.3
Coleoptera	55.5				

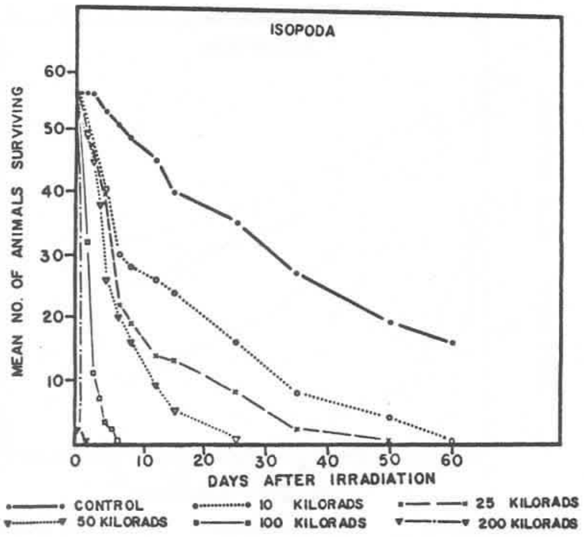

Fig. 5. Effects of different doses of gamma radiation on invertebrates in culture.

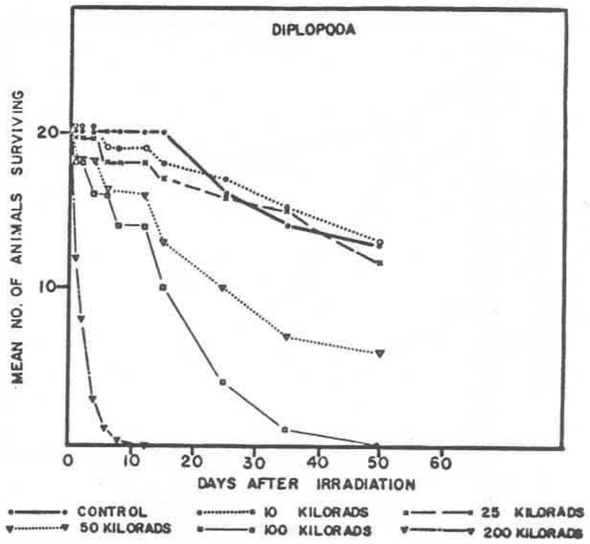

Fig. 6. Effects of different doses of gamma radiation on invertebrates in culture.

Table 2. Survival time in soil after treatment with 200 kilorads gamma radiation

Taxonomic Category	Minimum Time for 100% Mortality in Soil After Exposure to 200 Kilorads (days)
Isopoda	Less than 1
Chilopoda	
Collembola-Entomobryidae	10
Collembola-Sminthuridae	
Acarina-Parasitidae	20
Symphyla	
Pauropoda	
Diplopoda	
Thysanoptera	
Lumbricidae	
Enchytraeidae	30
Collembola-Isotomidae	40
Coleoptera adults	
Diptera larvae	
Nematoda	50
Collembola-Onychiuridae	70
Collembola-Poduridae	80
Acarina-Oribatidae	

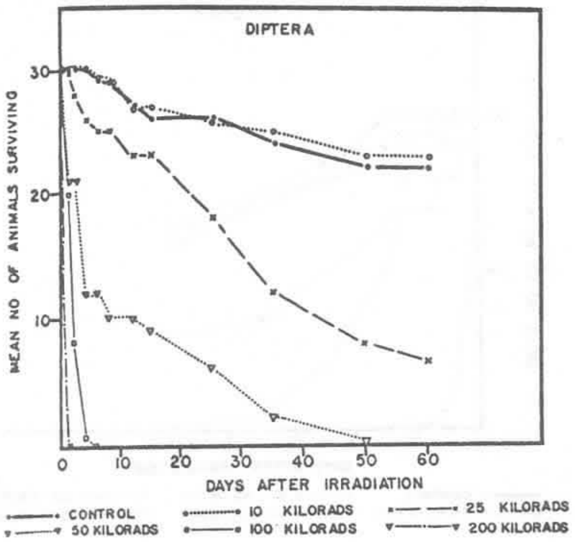

Fig. 7. Effects of different doses of gamma radiation on invertebrates in culture.

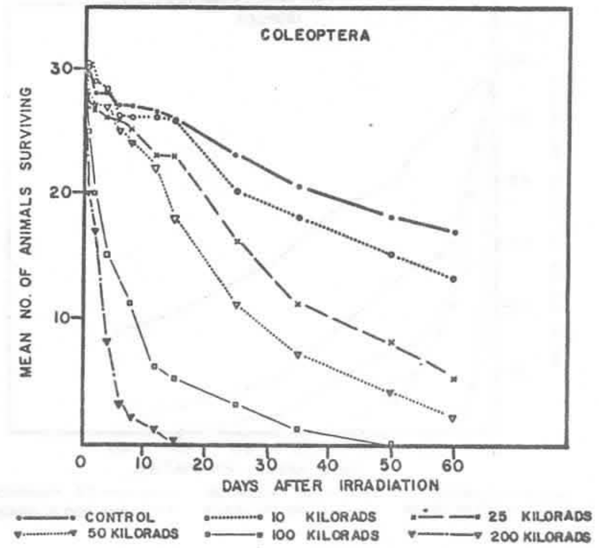

Fig. 8. Effects of different doses of gamma radiation on invertebrates in culture.

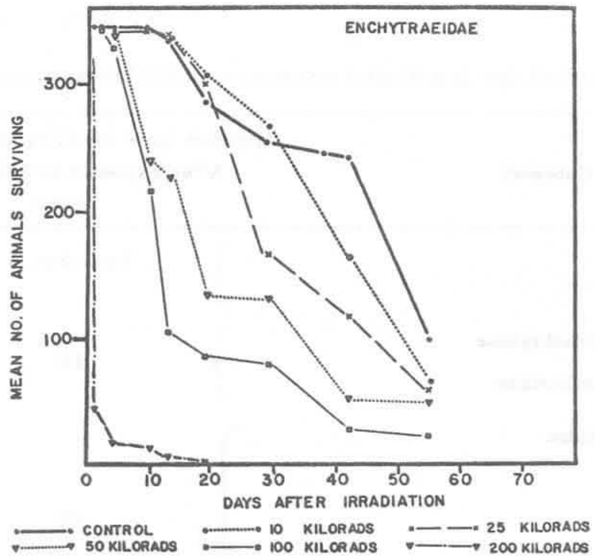

Fig. 9. Effects of different doses of gamma radiation on invertebrates in culture.

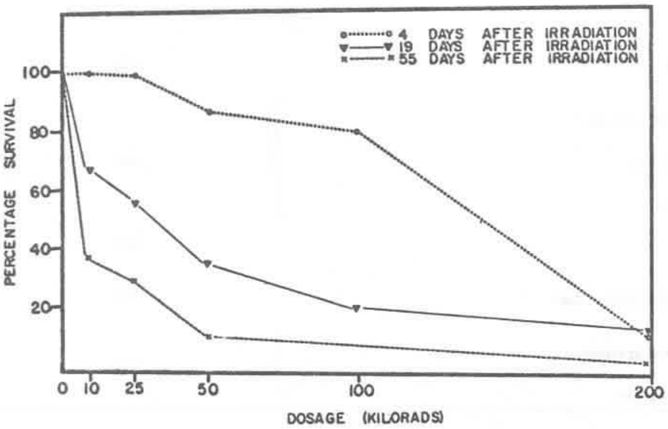

Fig. 10. Effects of dose rate on survival of all invertebrates in soil.

an underestimate. Hence, the figures quoted are the minimum times for 100% mortality. The relationship between survival time of the total population of invertebrates and radiation dose is summarized in Fig. 10.

Discussion

Most insects are killed by doses of gamma radiation in the range of one to fifty kilorads, although the eggs and immature stages may be susceptible to much lower doses. The investigations reported here show that other invertebrates have a similar range of susceptibility to radiation although some groups are more resistant. A dose of 200 kilorads was sufficient to kill all soil invertebrates in 80 days, and there was good evidence that a dose of 100 kilorads or less would ultimately eliminate most groups of invertebrates from soil. The susceptibilities of the common groups of small soil animals to a large dose of gamma radiation are compared in Table 2. The most striking feature of these data is the clear correlation between the activity and mobility of the animals and their susceptibility to the radiation. The more active animals, such as centipedes, wood lice, surface-dwelling Collembola (Entomobryidae and Sminthuridae), parasitic mites and symphylids were very much more susceptible to irradiation than the sluggish oribatid mites or deep soil-dwelling Collembola (Onychiuridae and Poduridae). Worms, nematodes, fly larvae and beetles are of intermediate activity and are also moderately susceptible to irradiation.

Irradiated soil invertebrates died sooner in plaster of Paris cultures than in intact soil samples stored at the same temperature. For example, although wood lice were killed in less than one day by 200 kilorads both in soil and in culture, millipedes survived for 8 days in culture and 20 days in soil after the same treatment. Enchytraeid worms survived for 19 days in culture and 40 days in soil, beetles for 15 days compared with 40 days in soil, onychiurid Collembola 50 days instead of 70 and oribatid mites 50 days instead of 80 days in soil. The most surprising results were, however, for the parasitic mites and dipterous larvae which were both killed in one day in the cultures by 200 kilorads, but in soil survived for 20 and 40 days respectively after treatment with the same dose. This may be that the species chosen to represent these two groups in the culture experiments were particularly susceptible to gamma radiation; certainly they were large and active species. The greater susceptibility of the culture animals is hardly surprising. They were living under unnatural conditions and in Table 2 only the time which the toughest individual of the most resistant species in the group required to die is recorded. Most species of invertebrates will be more susceptible to irradiation than this. The relative susceptibility of the various groups will be the same, however.

The pathological symptoms produced in the various groups of invertebrates by the radiation were varied and interesting. Animals from all groups showed a tendency to become much more sluggish and inactive after exposure to a large dose of radiation if it did not immediately kill or paralyze them. Individual responses to irradiation varied. In most groups, even when smaller doses were applied, some animals were dead immediately after treatment. At the other extreme, there were individuals that seemed resistant to very large doses. Paralysis was a common symptom and often lasted several days before death, with only periodic tremors of the legs and antennae to indicate life. This phase was most prolonged for the onychiurid Collembola, a few of these remaining paralyzed for two or three weeks before dying. None of the paralyzed animals ever recovered. Females of some species, in particular wood lice and millipedes, tended to lay eggs shortly before they died, but none of these were viable.

The relationship between dose and mortality was much more complex and variable than the relationship between insecticidal dose and mortality. This is not surprising because most insecticides have a single site of action, the stomach or nervous system, whereas gamma radiation is nonselective in its action; its effect varying only with the dose level and the susceptibilities of the different types of cell in the invertebrate body. Some invertebrates showed a steady increase in mortality as the radiation dose increased, e.g., oribatid mites (Fig. 4). By contrast, no uropod mites (Fig. 2) were killed until a threshold dose of 50 kilorads was applied; after this, a small increase in dose greatly increased mortality.

The mortality/time curves for the cultures at different dosages (Figs. 1–9) were very interesting. For most biocides mortality varies sigmoidally with time and approximates to a straight line when mortality is plotted on a probit scale and time is plotted on a logarithmic one. The gamma radiation data is plotted on linear scales in the figures, but when plotted on probit/log scales, there were two distinct phases; an initial stage complete for animals in all groups in six days or less, followed by a shallower secondary-mortality line that varied in slope for each group of animals and with the dosage of radiation applied. As the dose increased the number of animals killed in the first phase became greater. The mortality/time curves for *Tribolium confusum* (Cork 1967) clearly show two phases, but neither Cork nor other authors (Laviolette and Nardon 1963, Tilton *et al.* 1966) comment on this.

The corresponding data for the intact soil samples plotted as mortality/time curves also showed two distinct phases, and there were two phases to the mortality/dose curves when plotted on probit/log axes. There are several possible explanations for the form of these mortality curves; for example, possibly the animals in the cultures were heterogeneous in age or other factors were affecting their susceptibility to radiation. If this were so, it is unlikely that the two phases in the curves would be so consistent. A more probable theory is that there are two mechanisms of response to the radiation, the first producing a rapid or shock reaction which is complete in a few days. Those animals which escaped this damage would then succumb more slowly.

There is not space to give the full data for the effect of the doses of radiation on *populations* of different invertebrates in the soil samples, but Fig. 10 summarizes the effects of increasing dose rates on the survival of *all* the animals in the soil samples at a few representative time intervals. It clearly shows that treatment with 200 kilorads of gamma radiation greatly reduced the numbers of animals in soil even a few days after the irradiation. Seventy-six days after treatment with 200 kilorads, only three oribatid mites and one podurid Collembola remained alive compared with several thousand animals in the ten untreated control samples. It seems probable that the dose of radiation necessary to destroy all invertebrate animals and eggs in soil lies between 100 and 200 kilorads, but this may take several months. It could be much lower because although lower doses may not kill all the animals in soil, they may sterilize them and cause infertile mating so that the population eventually dies out. Single families or orders of invertebrates were completely eradicated by much lower doses as can be seen in Table 1. Such effects raise the question of the possible use of gamma irradiation as a technique for sterilizing soil for horticultural or other use. Although the packaging plant at Wantage could sterilize quantities of soil of the order of a few cubic yards, it would not be an economic proposition and the development of field methods of applying gamma radiation is even more difficult. It would involve the systematic dragging of a source and shielding, over land in order to obtain penetration to an effective depth, and as Cornwell and Bull (1960) observed, such operations could only be done by staff trained in handling of radiation sources and would be prohibitively expensive. As nuclear technology advances, however, such operations should not be excluded from the bounds of possibility.

The variation in susceptibility of different species of soil invertebrates to gamma irradiation may prove to be an invaluable tool in studying ecological interactions between various groups of animals in the terrestrial invertebrate community. It would be easy to choose a dose of radiation that would eradicate some groups of animals but leave others free to multiply reaching or increasing beyond their original numbers in a relatively short time. Rohde (1959) gave an example of differential susceptibility to radiation of a predator and its prey. He showed that the eggs of the parasitic mite *Fuscuropoda marginata* (C. L. Koch) were much more susceptible to gamma irradiation than those of its saprophagous prey *Caloglyphus mycophagus* (Megnin). If a dose of 50 kilorads is applied, probably the only animals living after several months would be oribatid mites, podurid and onychiurid Collembola.

The effects of the radiation on the populations of soil animals in the intact soil samples indicated the occurrence of this kind of interaction between predator and prey organisms. The parasitic mites were much more susceptible to the radiation than the isotomid, onychiurid and podurid Collembola and in the samples treated with 10 kilorads several species of isotomid and onychiurids increased considerably in numbers in the treated soil compared with the controls.

Although the animals in the samples have been sorted into families, the specific identifications are not yet complete; so, full details of the increases will be given in a future paper. Further investigations are being made into the effects of treatment with five kilorads to determine whether this dose will produce even more distinct increases in numbers of Collembola than the higher ones.

As Auerbach (1958) pointed out, we are still very ignorant of the soil ecosystem and on the long term effects of the contamination of this system with radioactive materials. It is certain that much more detailed study of both lethal and sub-lethal doses of radiation would be a fruitful field for ecological investigations and would greatly extend our knowledge of possible hazards and problems resulting from increases in the use of nuclear power and isotopes.

Acknowledgments

I wish to thank Mr. P. A. Cawse, Wantage Research Laboratory (AERE), Berks, England, for his advice and cooperation and for making radiation facilities available and Mr. A. E. Whiting of Rothamsted Experimental Station for considerable help with sorting the soil animals into taxonomic categories.

Appendix

Species of Animals Tested in Culture Experiments

Order	Family or Superfamily	Species
Collembola	Onychiuridae	*Onychiurus* sp.
Acarina	Uropodoidae	*Olodiscus minima*
	Parasitidae	*Alliphis halleri*
	Ceratozetoidae	*Punctioribates punctum*
Isopoda	Oniscoidae	*Porcellio scaber*
Diplopoda	Polydesmidae	*Polydesmus angustus*
Diptera	Stratiomyidae	*Pachygaster* sp.
Coleoptera		*Philonthus* sp.
Enchytraeidae		*Gen et sp.* unknown

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